

P19850.A10

Generation and Wound Healing", N. Engl. J. Med., 315:1650-1659 (1986), the disclosure of which is herein incorporated by reference in its entirety, and is likely mediated by similar and specific angiogenic molecules (e.g., VEGF), which are released by the tumor cells and/or host immune cells into the stroma or possibly mobilized from a bound inactive state within the tumor stroma (e.g., FGF-1), as disclosed in FOLKMAN et al. (1987); FOLKMAN (1995); and MOSCATELLI et al. (1981), all cited above, the disclosures of which are herein incorporated by reference in their entireties. In addition to tumor cells, inflammatory cells may also be important in tumor angiogenesis. Stimulated macrophage can secrete angiogenic factors, such as TGF α , angiotropin, TNF α , and bFGF/FGF-2, as disclosed in FOLKMAN (1995), cited above; POLVERINI et al., "Induction of Neovascularization *in vivo* and Endothelial Proliferation *in vitro* by Tumor Associated Macrophages", Lab. Invest., 51:635-642 (1984); BAIRD et al., "Immunoreactive Fibroblast Growth Factor in Cells of Peritoneal Exudate Suggests its Identity with Macrophage-derived Growth Factor", Biochem. Biophys. Res. Commun., 126:358-364 (1985); FRATER-SCHRODER et al., "Tumor Necrosis Factor Type α , a Potent Inhibitor of Endothelial Cell Growth *in vitro*, is Angiogenic *in vivo*", Proc. Natl. Acad. Sci (USA), 84:5277-5281 (1987); LEIBOVICH et al., "Macrophage-induced Angiogenesis Mediated by Tumour Necrosis Factor- α ", Nature, 329:630-632 (1987); SCHREIBER et al., "Transforming Growth Factor-alpha: a More Potent Angiogenic Mediator than Epidermal Growth Factor", Science, 232:1250-1253 (1986); HOCKEL et al., "Purified Monocyte-derived Angiogenic Substance (Angiotropin) Induces Controlled Angiogenesis Associated with Regulated Tissue Proliferation in Rabbit Skin", J. Clin. Invest., 82:1075-1090 (1988); and FOLKMAN et al., "A Heparin-binding Angiogenic Protein - Basic Fibroblast Growth Factor - is Stored within

P19850.A10

Basement Membrane", Am. J. Pathol., 130:393-400 (1988), the disclosures of which are herein incorporated by reference in their entireties.---

Please replace the original paragraph bridging pages 9-11, as previously modified by the July 16, 2001 Preliminary Amendment, with the following new paragraphs:

---Various low molecular weight, non-peptide angiogenic factors have also been reported. These include 1-butyryl-glycerol, prostaglandins E1 and E2 (PEG1 and PEG2), nicotinamide, adenosine, nitric oxide, hyaluronic acid degradation products, an arachidonic acid metabolites named 12(R)-hydroxyeicosatrienoic acid (12[R]-HETrE), 8Br-cAMP, estrogens (17 β -estradiol), as disclosed in FOLKMAN et al. (1987), cited above; FOLKMAN (1995), cited above; LEIBOVICH et al., "Production of Angiogenic Activity by Human Monocytes Requires an L-arginine/nitric oxide-synthase-dependent Effector Mechanism", Proc. Natl. Acad. Sci (USA), 91:4190-4194 (1994); LANIADO-SCHWARTZMAN et al., "Activation of Nuclear Factor $\kappa\beta$ and Oncogene Expression by 12(R)-hydroxyeicosatrienoic acid, an Angiogenic Factor in Microvessel Endothelial Cells", J. Biol. Chem., 269:24321-24327 (1994); BANERJEE, "Microenvironment of Endothelial Cell Growth and Regulation of Protein N-glycosylation", Indian J. Biochem. Biophys., 25:8-13 (1988); and BANERJEE et al., "Biphasic Estrogen Response on Bovine Adrenal Medulla Capillary Endothelial Cell Adhesion, Proliferation and Tube Formation", Mol. Cell Biochem., 177:97-105 (1997). When endothelial cells are stimulated by 12(r)-HETrE, the proto-oncogenes *c-myc*, *c-jun*, and *c-fos* are activated, as disclosed in LANIADO-SCHWARTZMAN et al. (1988), cited above, the disclosure of which is incorporated herein by reference in its entirety.

P19850.A10

Inactivation of a suppressor gene resulting in loss of an angiogenic suppressor substance may allow tumor angiogenesis to proceed. Indeed, the switch to active angiogenesis and the rate of the angiogenic process are likely the net effect of both stimulatory and inhibitory factors. For example, it has been shown that inactivation of a suppressor gene during carcinogenesis results in increased angiogenesis that parallels increased tumorigenicity, as disclosed in BOND et al., "Replacement of Residues of 8-22 of Angiogenin with 7-21 of RNASE-A Selectively Affects Protein-synthesis Inhibition and Angiogenesis", Biochemistry, 29:3341-3349 (1990); and BOUCK et al., "Coordinate Control of Anchorage Independence, Actin Cytoskeleton and Angiogenesis by Human Chromosome 1 in Hamster-human Hybrids", Cancer Res., 46:5101-5105 (1986), the disclosures of which are herein incorporated by reference in their entireties. During this process there is a 10-fold decrease in the secretion of an angiogenesis inhibitor 140 kDa glycoprotein, thrombospondin, as disclosed in RASTINEJAD et al., "Regulation of the Activity of a New Inhibitor of Angiogenesis by a Cancer Suppressor Gene", Cell, 56:345-355 (1989), the disclosure of which is herein incorporated by reference in its entirety.

Somatic hybrid cells produced by fusion of MCF-7 human breast carcinoma cells with normal immortalized human mammary epithelial cells are suppressed in their ability to form tumors in nude mice, as disclosed in ZAJCHOWSKI et al., "Suppression of Tumor-forming Ability and Related Traits in MCF - 7 Human Breast Cancer Cells by Fusion with Immortal Mammary Epithelial Cells", Proc. Natl. Acad. Sci (USA), 87:2314-2318 (1990), the disclosure of which is herein incorporated by reference in its entirety. The hybrids has among other traits of their normal parent cells, the ability to increase the expression of the angiogenesis inhibitor thrombospondin.

P19850.A10

A “switch” to the angiogenic phenotype by fibroblasts cultured from Li-Fraumeni patients coincides with loss of the wild-type allele of the p53 tumor suppressor gene and reduced expression of thrombospondin-1. A novel angiogenesis inhibitor, “angiostatin” is released by the primary tumor mass of a Lewis lung carcinoma. When the primary tumor is present, metastatic tumor growth is suppressed by “angiostatin”; but, after primary tumor removal, the metastases neovascularize and grow. The “angiostatin” activity co-purifies with a 38 kDa plasminogen fragment, as disclosed in O'REILLY et al., "Angiostatin: A Novel Angiogenesis Inhibitor that Mediates the Suppression of Metastases by a Lewis Lung Carcinoma", Cell, 79:315-328 (1994), the disclosure of which is herein incorporated by reference in its entirety. Similarly, endostatin a 20 kDa C-terminal fragment of collagen XVIII prevents the angiogenic switch in pre-malignant lesions, intervening in the rapid expansion of small tumors, or inducing the regression of a large end-stage cancers, as disclosed in O'REILLY et al. (1994), cited above; and BERGERS et al., "Effects of Angiogenesis Inhibitors on Multistage Carcinogenesis in Mice", Science, 284:808-812 (1999), the disclosures of which are herein incorporated by reference in their entireties. Other negative regulators of endothelial proliferation include: platelet factor 4, tissue inhibitors of metalloproteinases, a 16 kDa fragment of prolactin, bFGF/FGF-2 soluble receptor, and TGF β , as disclosed in FOLKMAN (1995), cited above, the disclosure of which is herein incorporated by reference in its entirety.---

Please replace the original paragraph bridging pages 24-26, as previously modified by the July 16, 2001 Preliminary Amendment, with the following new paragraphs:

---Activation of mammalian *UPR* is characterized in part by increased transcription of at least seven genes encoding ER molecular chaperons. These are Bip/GRP78, as disclosed in LEE, "Mammalian Stress Response: Induction of the Glucose-regulated Protein Family", Curr. Opin. Cell Biol., 4:267-273 (1992), the disclosure of which is herein incorporated by reference in its entirety, as well as induction of C/EBP homologous protein (CHOP), a transcription factor also known as growth arrest and DNA damage gene product-153 or GADD153, as disclosed in WANG et al., "Signals from the Stressed Endoplasmic Reticulum Induce C/EBP-homologous Protein (CHOP/GADD153)", Mol. Cell. Biol., 16:4273-4280 (1996); and WANG et al., "Cloning of Mammalian Ire1 Reveals Diversity in the ER Stress Responses", EMBO J., 17:5708-5717 (1998), the disclosures of which are herein incorporated by reference in their entireties. Three ER transmembrane signaling proteins that are thought to be the proximal effectors of the *UPR* are Ern1 and 2, PERK, as disclosed in WANG et al., "Cloning of Mammalian Ire1 Reveals Diversity in the ER Stress Responses", EMBO J., 17:5708-5717 (1988); TIRASOPHON et al., "A Stress Response Pathway from the Endoplasmic Reticulum to the Nucleus Requires a Novel Bifunctional Protein Kinase/Endoribonuclease (Ire1p) in Mammalian Cells", Genes Dev., 12:1812-1824 (1998); and HARDING et al., "Protein Translation and Folding are Coupled by an Endoplasmic-reticulum-resident Kinase", Nature, 397:271-274 (1999), the disclosures of which are herein incorporated by reference in their entireties.

In principle, the mechanism underlying *UPR*-induced ER-stress condition could indirectly impede cell-cycle progression by interfering with the proper maturation of growth factor receptors or other modulators of mitogenic signaling, as disclosed in CAI et al., "Down-Regulation of

P19850.A10

Epidermal Growth Factor Receptor-Signaling Pathway by Binding of GRP78/BiP to the Receptor Under Glucose-Starved Stress Conditions", Journal of Cellular Physiology, 177:282-288 (1998), the disclosure of which is herein incorporated by reference in its entirety. Alternatively, ER stress may directly induce checkpoint response that prevents cells from completing their cell division cycle under conditions that compromise the proper folding and assembly of proteins response, as disclosed in BREWER et al., "Mammalian Unfolded Protein Response Inhibits Cyclin D1 Translation and Cell-cycle Progression", Proc. Natl. Acad. Sci (USA), 96:8505-8610 (1999); and NAKAGAWA et al., "Caspase-12 Mediates Endoplasmic-reticulum-Specific Apoptosis and Cytotoxicity by Amyloid- β ", Nature, 403:98-103 (2000), the disclosures of which are herein incorporated by reference in their entireties. Since the late 1970s there has been a clear link between sugar metabolism and the *UPR*, as disclosed in POUYSSSEGUR et al., "Induction of Two Transformation-sensitive Membrane Polypeptides in Normal Fibroblasts by a Block in Glycoprotein Synthesis or Glucose Deprivation", Cell, 11:941-947 (1977); SHIU et al., "Glucose Depletion Accounts for the Induction of Two Transformation-sensitive Membrane Proteins in Rous Sarcoma Virus-transformed Chick Embryo Fibroblasts", Proc. Natl. Acad. Sci. (USA) 74:3840-3844 (1977); and PELUSO et al., "Infection with Paramyxoviruses Stimulates Synthesis of Cellular Polypeptides that are also Stimulated in Cells Transformed by Rous Sarcoma Virus or Deprived of Glucose", Proc. Natl. Acad. Sci. (USA), 75:6120-6124 (1978); GETHING et al., "Protein Folding in the Cell", Nature, 355:33-45 (1992); PAHL et al., "A Novel Signal Transduction Pathway from the Endoplasmic Reticulum to the Nucleus is Mediated by Transcription Factor NF-kappa B", EMBO J., 14:2580-2588 (1995); and WATOWICH et al., "Complex Regulation of Heat Shock- and Glucose-responsive Genes in Human